THE EFFECT OF HYPERTONIC SUGAR SOLUTIONS ON THE THERMAL RESISTANCE OF BACTERIA[1]

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INTRODUCTION

In connection with the various studies on the bacteriology of ice cream which have been in progress at the Kansas Agricultural Experiment Station for several years, it has been observed occasionally that micro-organisms exhibit an increased thermal resistance when heated in ice-cream mix. Preliminary experiments showed quite definitely that certain strains of bacteria were capable of withstanding more severe heat treatment when suspended in solutions of high osmotic pressure.

The temperature and time of exposure most commonly employed in the pasteurization of ice-cream mix have been adopted from the market-milk industry without question as to the universality of their application. If thermal resistance is affected by the chemical and physical forces of the environment it is entirely logical to expect a variation in the survival of cells heated in different menstrua. The small margin of safety in the present requirements for pasteurization emphasizes the importance of evaluating any increase in thermal resistance of the microflora which may be contributed by the ingredients of ice cream.

REVIEW OF LITERATURE

Until comparatively recently the protective action of the ingredients of ice-cream mix for micro-organisms has not been recognized. Beavens (*4*) [2] found that 4- to 20-percent lactose increased the thermal resistance of *Escherichia coli*. The probability that the ingredients of ice cream might afford micro-organisms some protection against heat was recognized by the Committee on Dairy Products and Eggs (*15*). The results of Oldenbusch, Frobisher, and Shrader (*8*) gave only slight evidence of increased survival of various pathogenes when heated in ice-cream mix or in cream containing 50 percent fat. Fabian and Coulter (*6*) observed higher thermal death points for cultures of *E. coli* and *Aerobacter aerogenes* when heated in ice-cream mix than when heated in skim milk. Except for sucrose, these authors were unable to show any marked protective effect when the ingredients of ice cream were studied separately.

Anzulovic (*1*) reported that sugar, gelatin, serum solids, and fat showed some protective action for bacteria. Weiss (*17*) found that *Bacillus botulinus* was more resistant to heat in foods containing heavy sirups. Rahn (*9*) stated that—

[1] Received for publication Oct. 17, 1933; issued May 1934. A résumé of a dissertation submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy. The work was made possible through the cooperation of the Department of Dairy Industry, Iowa State College of Agriculture and Mechanic Arts, and the Department of Bacteriology, Kansas State College of Agriculture and Applied Science. Published as doctoral dissertation no. 244, Iowa State College, and contribution no. 154 of the Department of Bacteriology, Kansas State College.

[2] Reference is made by number (italic) to Literature Cited, p. 467.

* * * sugar not only retards growth of yeasts and other micro-organisms, but also retards the action of heat upon micro-organisms; it will take more heat to kill a bacterium or yeast in a sweetened fruit juice than in the same juice without sugar.

Robertson (*11, 12, 13, 14*) heated *Streptococcus thermophilus*, *Sarcina lutea*, *Escherichia coli*, and *Micrococcus aureus* in increasing percentages of sugar and found that as the concentration of sugar was increased the number of surviving bacteria also increased.

Nechkovitch (*7*) showed that glucose tended to prevent the coagulation of cell colloids and aided in maintaining a normal condition of stability of the cells of organized tissue. Wallace and Tanner (*16*) suspended several species of molds in 10-, 25-, and 50-percent sugar, distilled water, sirups from fruit juices, and in salt water. Protective action was afforded by sugar for some molds and by salt water for others.

Rahn (*10, p.330*) reported increased thermal resistance of yeasts and bacteria when heated in broth containing 50 percent of sucrose. This author suggested that although death may be due in part to dehydration, the cause of death is not the same as with dry bacteria. Cook[3] explained his observations of increased resistance of yeasts in hyper tonic glucose and sucrose solutions on the basis of dehydration of the cells. He stated, however, that there was probably some factor other than osmotic pressure involved, since the killing times were not proportional to the osmotic pressure of the solutions employed.

Beilinsson (*5*) observed that the addition of sufficient sucrose or glycerol to serum albumin or to egg white rendered these proteins stable to temperatures far above the usual coagulation points. Bancroft and Rutzler (*3*) and Bancroft and Richter (*2*) confirmed these observations and suggested an explanation based upon the peptizing action of sugar on albumin. According to these authors, if the colloidal suspension is reversibly coagulated, the cell may have lost temporarily any or all of its vital manifestations, but will recover from dormancy when placed in a favorable environment. As agglomeration of the cell contents increases, the cell loses more and more of its functions, the coagulation becomes progressively less reversible and finally completely irreversible.

METHODS

In all the plating procedures standard beef-extract agar was employed as the basic medium. In those instances in which comparisons were made between the counts on plain and carbohydrate media, a large batch of the plain agar was divided into equal parts and 1 percent of the desired carbohydrate added to each portion. The reaction of all media was adjusted to pH 7.0 before filtration.

The distilled water used in making dilution blanks occasionally was tested after sterilization for its reaction and found to be pH 6.0 ± 0.2.

All plates were incubated 48 hours at 37° C. In several of the experiments where delayed germination was suspected, counts were made again after an additional 3 days' incubation at room temperature.

In several experiments reference is made to the use of a 100-percent sugar solution. This refers to a weight-volume ratio, and the solution was prepared in the following manner: Approximately 250 cc of water was added to 1 kg of the desired sugar, and this was boiled for a few

[3] COOK, W. B. STUDIES ON THE STERILIZATION OF SOLUTIONS OF GLUCOSE AND SUCROSE. (Thesis, Ph.D., Iowa State College.) 1931.

minutes until a clear solution was obtained. The resulting sirup (approximately 900 cc) was diluted to 1,000 cc total volume. Such a solution therefore contained 1,000 g of sugar in a total volume of 1,000 cc, and each cubic centimeter represented 1 g of sugar. All the other sugar solutions employed, the concentrations of which are expressed in percentages, were prepared by suitable dilution of this 100-percent sugar solution. The sugar solutions, the concentrations of which are expressed in molality, were prepared by dissolving the indicated number of gram-molecular weights in 1,000 cc of water.

In order to reduce the factor of heat penetration to a practical minimum, small samples were used in all heating trials. In some cases, 1.5 to 2.0 cc samples were placed in small hermetically sealed tubes and completely submerged in a water or an oil bath for the desired heating period. In other cases, thin-walled, small-bore (4 mm) test tubes were submerged in the oil bath to within 1 inch of the top of the tube. For many of the experiments special tubes were prepared by blowing a bulb about 1.5 inches in diameter on the end of a soft glass test tube. The small sample in the relatively large bulb of very thin glass acquired the temperature of the water bath very quickly. Obviously, heat penetration would be delayed in samples containing high percentages of sugar. However, the importance of this factor was shown to be reduced to a negligible minimum when small samples (2 cc or less) were employed. Graphs illustrating the rates of heat penetration under the conditions of these experiments show practically superimposed lines.

Although the results of the preliminary experiments did not indicate that it was necessary, all tubes containing more than a 0.1 cc sample were agitated by uniform shaking during the heating period.

RESULTS

HYPERTONIC SOLUTIONS IN ICE CREAM MIX

A sample of ice cream mix which contained no sucrose was incubated at room temperature until the bacterial count reached several million per cubic centimeter. This was divided into four parts and equal volumes of sterile sucrose solutions were added to give 15-, 25-, and 50-percent concentrations of sugar. A similar volume of water was added to one portion, which was designated as 0-percent sucrose. Plates were made before and after heating small portions of the four samples simultaneously to 54.5° C. for 9 minutes. In order to facilitate comparison, the number of viable organisms after heating has been calculated on the basis of survival per million.

The data in table 1 were compiled from the results of four such trials, and illustrate the general trend of many similar experiments. It will be observed in experiment 1 that the ice cream mix had a plain agar plate count of 14,000,000 per cubic centimeter before heating. After heating, the survival per million was 450 in the sample containing no sucrose, and 35,000, 44,000, and 35,000 in the samples containing 15-, 25-, and 50-percent concentrations of sucrose, respectively. Since all four samples were identical except for the sugar, the results suggest that the presence of sugar in ice cream renders the cells more resistant to heat.

TABLE 1.—*The effect of plain and carbohydrate agars on the survival of micro-organisms in ice cream mix containing various amounts of sugar after heating at 54.5° C. for 9 minutes*

Experiment no.	Agar	Count per cubic centimeter before heating	Survival per million after heating in ice cream mix containing—			
			0 percent sucrose	15 percent sucrose	25 percent sucrose	50 percent sucrose
		Millions	*Number*	*Number*	*Number*	*Number*
1	Plain	14	450	35,000	44,000	35,000
2	Plain	170	310	1,500	2,800	25,000
	Dextrose	180	19,000	14,000	14,000	61,000
	Sucrose	130	24,000	18,000	22,000	77,000
	Lactose	170	19,000	18,000	22,000	59,000
3	Plain	110	220	5,500	5,900	44,000
	Sucrose	110	5,500	8,000	8,000	39,000
4	Plain	66	260	7,900	7,900	18,000
	Sucrose	60	17,000	18,000	17,000	18,000

In the other experiments reported in table 1 the same routine was followed except that various carbohydrate agars were employed in pouring parallel plates from dilution blanks. In experiment 2, for example, the survival per million in the sample containing no sucrose was 310 when plated on plain agar, and from 19,000 to 24,000 on carbohydrate agars. This at once suggests that some of the cells, injured but not irreparably destroyed by the heat, were capable of recovery when the medium was fortified with a carbohydrate. In the samples containing 15- and 25-percent sucrose the plain agar counts per million were 1,500 and 2,800, respectively, which indicates that the presence of sugar in the sample diminished the degree of injury to the cells and enabled more of them to recover even in plain agar. Transfers from the same dilution blank to carbohydrate media gave survival per million values ranging from 14,000 to 22,000, again emphasizing the importance of the medium in the recovery of injured cells.

The protective action of sugar is most effectively illustrated in the samples containing 50 percent of sucrose. The thermal resistance of the cells was greatly increased in the presence of such excessive amounts of sucrose.

A consideration of the results of experiments 3 and 4 leads to the same general observations, viz, in ice-cream mix without sugar injury of the cells by heat is greater than when sugar is present; the number of organisms capable of surviving increases with increasing percentages of sugar in the mix (this is especially noticeable when 50 percent of sugar is employed); and more of the injured cells survive in carbohydrate media.

If high osmotic pressures result in an increased thermal resistance for cells, it is conceivable that micro-organisms whose normal resistance is at the threshold of the thermal exposure of pasteurization may survive in ice cream and not in milk.

HYPERTONIC SOLUTIONS IN MILK

Sterile milk was heavily inoculated with a pure culture of *Escherichia coli* 57 and then diluted (1) with an equal volume of water, and (2) with an equal volume of 100-percent sucrose, thereby giving an ultimate concentration of 50-percent sugar. These two samples were plated before and after heating (54.5° C., 9 minutes) on plain agar and on 1-percent dextrose, sucrose, and lactose agars.

The results in table 2 show that approximately 5 to 10 times as many organisms survived in the milk to which sucrose was added. In this experiment the use of carbohydrate agar did not increase the number of survivors—an observation that tends to discourage the conclusion that the injured cells are necessarily rendered more saccharophilic.

TABLE 2.—*Effect of various media on the recovery of Escherichia coli 57 heated at 54.5° C. for 9 minutes in suspensions of milk and milk containing 0- and 50-percent sucrose*

Medium	Count per cubic centimeter before heating		Survival per million after heating at 54.5° C. for 9 minutes	
	0-percent sucrose	50-percent sucrose	0-percent sucrose	50-percent sucrose
Plain	10,000,000	10,000,000	48,000	250,000
Dextrose	14,000,000	11,000,000	20,000	230,000
Sucrose	10,000,000	11,000,000	18,000	245,000
Lactose	12,000,000	15,000,000	30,000	150,000

HYPERTONIC SOLUTIONS IN BROTH

PROTECTIVE ACTION OF VARIOUS SUGARS FOR ESCHERICHIA COLI HELD FOR VARIOUS LENGTHS OF TIME

Uniform suspensions of *Escherichia coli* 25 were prepared in plain broth and in broths containing 50 percent, respectively, of maltose, sucrose, and dextrose. Because of the low solubility of lactose, suspensions of cells in saturated lactose broth were prepared separately. These suspensions were plated as quickly as possible on plain agar before and after heating to 54° C. for 9 minutes. After the original suspensions had stood at room temperature for 2 hours, plates were again made before and after heating to 54° for 9 minutes.

Table 3 shows that saturated lactose failed to protect *Escherichia coli* 25, and that 50-percent maltose broth afforded only very slight protection. The survival per million values in 50-percent sucrose and in 50-percent dextrose broths were increased ninefold and sevenfold, respectively, after the cultures had aged 2 hours.

TABLE 3.—*Thermal resistance of Escherichia coli 25 after 2 hours' contact with plain broth, saturated lactose broth, and 50-percent maltose, sucrose, and dextrose broths*

Cell suspension	Count per cubic centimeter after—				Survival per million after—	
	0 hour contact		2 hours contact			
	Before heating	After heating at 54° C. for 9 minutes	Before heating	After heating at 54° C. for 9 minutes	0 hour contact	2 hours contact
Plain broth	500,000	50	550,000	730	100	1,330
Saturated lactose broth	500,000	30	520,000	0	60	0
50-percent maltose broth	500,000	1,100	130,000	380	2,200	2,900
50-percent sucrose broth	340,000	9,800	220,000	58,000	29,000	264,000
50-percent dextrose broth	600,000	18,000	60,000	13,000	30,000	217,000

EFFECT OF PROLONGED EXPOSURE OF CELLS TO HYPERTONIC SUCROSE SOLUTION

Suspensions of *Escherichia coli* 52 in plain and 50-percent sucrose broths were held at 30° C. Samples were removed from the sucrose suspension after each 15-minute interval for 2 hours and then at less frequent intervals for 7 hours. Each sample removed was plated on plain agar before and after heating at 54.5° for 5 minutes.

A study of the results presented in table 4 shows a marked increase in the thermal resistance during the first 2 hours. It is significant to note that although the counts on the sucrose broth before heating were fairly constant, the actual number of cells capable of surviving the heat treatment increased. Evidently the physical changes responsible for greater heat stability of the protoplasm affect an increasing number of cells with time. In this instance the maximum number of cells capable of withstanding the heat treatment occurred after 2 hours' exposure, whereas continued contact with the sugar resulted in a decrease in the thermal resistance.

TABLE 4.—*The thermal resistance of Escherichia coli 52 after prolonged incubation at 30° C. in plain and in 50-percent sucrose broth before heating at 54.5° C. for 5 minutes*

Period of contact before heating (minutes)	Count per cubic centimeters in—				Survival per million in—	
	Plain broth		50-percent sucrose broth			
	Before heating	After heating at 54.5° C. for 5 minutes	Before heating	After heating at 54.5° C. for 5 minutes	Plain broth	50-percent sucrose broth
0	1,500,000	10	1,700,000	42,000	7	25,000
15			1,700,000	170,000		100,000
30			1,800,000	450,000		250,000
45			2,200,000	570,000		259,000
60	2,600,000	0	2,300,000	750,000	0	326,000
75			1,700,000	870,000		512,000
90			1,800,000	960,000		533,000
105			1,700,000	690,000		406,000
120	4,100,000	0	1,400,000	1,100,000	0	786,000
180	38,000,000	0	2,900,000	550,000	0	190,000
240	150,000,000	0	1,200,000	350,000	0	292,000
300	220,000,000	10	1,100,000	210,000	0	191,000
420	330,000,000	0	930,000	57,000	0	61,000

The values for the survival per million in the 50-percent sucrose broth have been plotted against time in figure 1. The graph clearly shows the tendency for the cells to become progressively more resistant to heat when exposed to hypertonic sucrose solutions for 2 hours and the tendency to lose this faculty on continued exposure.

These results suggest that high concentrations of sucrose may induce a physical change which no doubt is regulated by the permeability of the individual cell. This process, although ultimately leading to the death of the cell, at first increases the stability of the protoplasm to heat. Continued exposure, however, advances the degree of coagulation to a point beyond which plain agar can no longer induce peptization. The ultimate decline in the number of cells capable of surviving the heat treatment apparently is the result of prolonged desiccation beyond the point of optimum stability.

HYPERTONIC SOLUTIONS IN WATER

EFFECT OF VARIATIONS ON OSMOTIC PRESSURE

Escherichia coli 52 was suspended directly in hypertonic solutions of dextrose and sucrose of various molalities. One tenth cubic centimeter of an 18-hour plain-broth culture was placed in 5-cc quantities of the various sugar solutions; after thorough agitation these were allowed to stand at room temperature for 5 minutes. Two cubic centimeter portions of each suspension were then heated to 55° C. for 5 minutes. Plate counts were made on plain and dextrose agars before and after heating. The data, calculated to a basis of survival per million, are presented in table 5. Owing to the fact that each solution was inoculated separately, there was some unavoidable variation in the initial number of organisms in the solutions

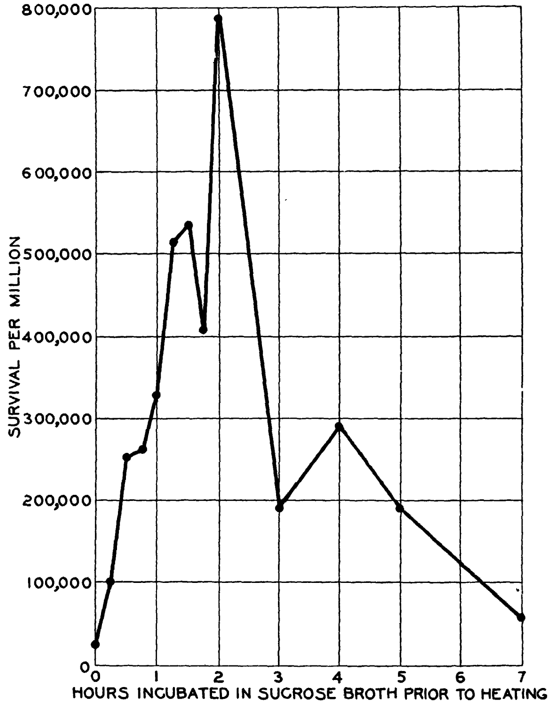

FIGURE 1.—Thermal resistance of *Escherichia coli* 52 after prolonged incubation at 30° C. in 50-precent sucrose broth before heating at 54.5° C. for 5 minutes.

TABLE 5.—*Survival per million of Escherichia coli 52 when heated in equimolal dextrose and sucrose solutions*

Molality of sugar solutions	Survival per million of *E. coli* when heated in—				Molality of sugar solutions	Survival per million of *E. coli* when heated in—			
	Dextrose solutions		Sucrose solutions			Dextrose solutions		Sucrose solutions	
	Plain agar	Dextrose agar	Plain agar	Dextrose agar		Plain agar	Dextrose agar	Plain agar	Dextrose agar
0.25	0	0	180	0	1.5	4,700	12,000	170,000	210,000
0.50	0	450	0	180	2.0	15,000	15,000	360,000	330,000
0.75	0	600	270	21,000	3.0	47,000	31,000	270,000	320,000
1.0	90	360	11,000	100,000	4.0	150,000	100,000	150,000	190,000

Since the osmotic pressures of the equimolal solutions were identical the differences observed in the protective action cannot be accounted for on this basis. It is possible, however, that there were differences in the effective osmotic pressure at the cell surfaces, since this would depend upon the permeability of the individual cell membrane. These results are in harmony with those of Cook[4] to which reference has already been made.

It is evident from table 5 that very little protective action is afforded by either sugar in concentrations below 1 molal. The values in table 5 show quite forcibly that there is an increased protective action with the increasing osmotic pressure beyond 1-molal concentrations.

Effect of Adding Sugar After Heating

In order to determine whether the increased survival of cells heated in hypertonic sugar solutions was attributable to the sugar carried over from the sample into the medium, the following experiment was performed. A 24-hour plain broth culture of *Escherichia coli* 52 was diluted with an equal volume of water in one tube, and with an equal volume of 100-percent sucrose solution in another. These cultures were heated to 54.5° C. for 5 minutes. After heating, the water suspension was divided into two equal parts; an equal volume of 100-percent sucrose solution was added to one part, and the other was similarly diluted with water. This provided (1) cells which had been heated in water and then the sugar added (50-percent concentration) after heating, and (2) cells heated in water and subsequently further diluted with water. In a similar manner the original suspension of cells which had been heated in 50-percent sucrose solution was divided into two parts, one of which was diluted with an equal volume of 50-percent sucrose solution and the other with an equal volume of water. If cells suspended in sucrose surround themselves with a layer of sugar which ultimately serves as an intimate source of energy in the agar plate, one would expect the suspension to which sugar was added after heating to exhibit approximately the same survival as the cells heated in the presence of sugar.

Table 6.—*Effect of adding 50-percent sucrose to cell suspensions of Escherichia coli 52 after heating at 54.5° C. for 5 minutes*

[Controls suspended in water]

Treatment of cells	Agar	Count per cubic centimeter		
		Before heating	After heating at 54.5° C. for 5 minutes [a]	Survival per million
Heated in 50-percent sucrose, then equal volume of 50-percent sucrose added.	Plain	78,000,000	2,600,000	67,000
	Dextrose	80,000,000	6,700,000	168,000
Heated in water, then equal volume o. 50-percent sucrose added.	Plain	190,000,000	300,000	3,200
	Dextrose	230,000,000	260,000	2,300
Heated in water, then equal volume of water added.	Plain	190,000,000	2,100,000	22,000
	Dextrose	230,000,000	1,100,000	10,000

[a] Since the samples were diluted with equal volumes of sucrose solution or water after heating, the values in this column should be doubled. The values for survival per million have been adjusted accordingly.

[4] Cook, W. B. See footnote 3.

The results in table 6 show that the addition of sugar after the cells had been heated in water failed to induce recovery from injury; in fact, the values for survival per million were actually smaller than those observed in the water suspension. The damage to the cells heated in water apparently cannot be counteracted by the subsequent addition of sugar. If the sugar is present during the heating a relatively large number of the cells are protected from irreparable injury.

EFFECT OF HYPERTONIC SUGAR SOLUTIONS ON THERMAL RESISTANCE OF VARIOUS BACTERIA

Some of the results already presented suggest that the permeability of the individual cell membrane may play an important part in the phenomenon of protective action. The results of several experiments not presented in this paper have suggested further that the protective action of dextrose and sucrose solutions was best demonstrated with organisms which were sensitive to heating in water. Considering these individual variations one would not expect, therefore, that all bacteria would be protected by hypertonic solutions.

The protective action of dextrose and maltose was tested on pure cultures of several different organisms. One cubic centimer of an 18-hour plain broth culture of the test organism was inoculated into each of three flasks containing 40 cc of water, 2-molal dextrose, and 2-molal sucrose, respectively. After thorough agitation samples were removed for plating and heating. The time of heating was 5 minutes in all cases, but the temperature employed varied with the different organisms.

TABLE 7.—*Effect of hypertonic solutions on the thermal resistance of cultures of several different organisms*

Culture	Count per cubic centimeter before heating in—			Temperature of exposure for 5 minutes	Count per cubic centimeter after heating in—		
	Distilled water	2-molal dextrose	2-molal sucrose		Distilled water	2-molal dextrose	2-molal sucrose
				° *C.*			
Serratia marcescens	28,000,000	23,000,000	17,000,000	55	100	540,000	160,000
Staphylococcus albus	40,000	16,000	60,000	55	0	0	0
Pseudomonas fluorescens	30,000	1,000	12,000	55	10	60	9,000
Aerobacter aerogenes	12,000,000	14,000,000	10,000,000	60	2,800	35,000	75,000
Staphylococcus aureus	13,000,000	37,000	7,900,000	60	120	190	2,600
Bacillus subtilis	130,000	130,000	140,000	90	20	130	500
Salmonella pullorum	1,000,000	1,500	1,000	55	190,000	1,100	1,200

The data in table 7 show striking variations in the effect of water, 2-molal dextrose, and 2-molal sucrose on thermal resistance of cells. First, it will be noted that there is little or no evidence that either dextrose or sucrose afforded the strains of *Staphylococcus albus* or *Salmonella pullorum* any increased resistance to heat. However, it should be noted that the counts before heating in the dextrose and sucrose suspensions of *S. pullorum* are practically the same as those obtained after heating. When compared with the counts on the aqueous suspensions before heating it is evident that very large percentages of the organisms died almost instantly when introduced into the sugar solutions, but those which did survive were able to withstand the heat treatment.

There is only slight evidence that 2-molal sucrose protected *Bacillus subtilis*, whereas the results with *Pseudomonas fluorescens* and *Staphylococcus aureus* indicate protective action more definitely. *Serratia marcescens* and *Aerobacter aerogenes* were unmistakably protected by the presence of either dextrose or sucrose. Two-molal dextrose apparently offered more effective protection to *S. marcescens* than did an equimolal solution of sucrose; the reverse was true, however, for *A. aerogenes*. In each case in which protective action was observed the cells exhibited extreme sensitivity to heat in water suspensions.

Effect of Washing Cells After Exposure to Sugar

Five cubic centimeters of an 18-hour plain broth culture of *Escherichia coli* 52 were centrifuged and all but 1 cc of the supernatant broth removed. One cubic centimeter of 100-percent sucrose was added and the resulting 50-percent sugar suspension of cells allowed to stand at room temperature for 30 minutes. The cells were then washed seven times with 0.85-percent NaCl solution to remove the sugar. At the appropriate time two 5-cc portions of a broth culture of the same organism were centrifuged to concentrate the cells. All but 1 cc of the supernatant broth was removed from one of these tubes and 1 cc of the 100-percent sucrose added as before. The cells were allowed to remain in contact with the sugar for 30 minutes at room temperature. The other tube of centrifuged cells was used as a broth control. In this case all but 2 cc of the supernatant broth was removed and the cells resuspended in the 2 cc volume of broth. After 1-cc portions of these three suspensions had been removed for plating before heating, the tubes were placed in the water bath at 54.5° C. for 5 minutes. Plates before and after heating were made with plain and dextrose agars.

TABLE 8.—*Thermal resistance of Escherichia coli 52 when exposed to 50-percent sucrose 30 minutes, then washed in 0.85-percent NaCl*

Treatment of cells	Agar	Count per cubic centimeter— Before heating	Count per cubic centimeter— After heating at 54.5° C. for 5 minutes	Survival per million
Exposed to 50-percent sucrose 30 minutes, washed 7 times, and heated in 0.85-percent saline.	Plain	280,000,000	340,000	1,200
	Dextrose	320,000,000	410,000	1,300
Exposed to 50-percent sucrose 30 minutes, not washed, heated in the sucrose solution.	Plain	840,000,000	400,000,000	476,000
	Dextrose	900,000,000	400,000,000	444,000
Centrifuged from broth, resuspended, and heated in broth.	Plain	1,000,000,000	1,300,000	1,300
	Dextrose	960,000,000	4,200,000	4,400

It is quite evident from the data in table 8 that the protective action which 50-percent sugar afforded cells was readily removed by washing the cells in an 0.85-percent NaCl solution. The cells heated in saline and in broth suspensions were quite susceptible to heat, whereas the presence of sugar during heating resulted in increased resistance.

PROTECTIVE ACTION OF HYPERTONIC SOLUTIONS AGAINST THE COAGULATION OF NONLIVING PROTEIN SYSTEMS

It is obviously difficult to demonstrate changes in the physical status of the colloidal system of a minute bacterial cell except by indirect means. If the coagulation of cell colloids follows simple, well-defined laws of colloid chemistry, then the protective action afforded by sugars should be readily demonstrable with nonliving colloids. The following experiments were designed to investigate such a possible parallelism.

Effect of Hypertonic Dextrose and Sucrose Solutions on the Coagulation of Egg Albumin

By serial dilution of 8-molal sucrose and dextrose solutions, 6-, 4-, 3-, 2-, 1-, 0.5-, 0.2-, and 0.1-molal concentrations of each sugar were prepared. From these, series of tubes were arranged each containing 1 cc of one of the foregoing concentrations of sugar. To each tube 1 cc of fresh egg albumin was added and thoroughly mixed with the sugar solution, thus reducing the sugar to one half its original concentration. Tubes containing a mixture of equal parts of water and egg albumin, and also tubes contained undiluted egg albumin were used as controls.

The series of tubes for both sugars were placed simultaneously in a water bath at the desired temperature and the time of coagulation noted. It was necessary to adopt an arbitrary standard of turbidity for coagulation in order that the readings might be rendered uniform. The albumin was arbitrarily regarded as coagulated when it was no longer possible to identify and differentiate the letters on a typewritten page held behind the tube; the diameter of the tube was 10 mm.

Table 9.—*Effect of equimolal concentrations of dextrose and sucrose on the coagulation of egg albumin*

Molality of sugar	Time required to coagulate egg albumin at—					
	60° C.		65° C.		100° C.	
	Dextrose	Sucrose	Dextrose	Sucrose	Dextrose	Sucrose
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
4	([a])	([a])	([a])	([a])	0.5	0.5
3	([a])	([a])	34	52	.2	.2
2	73	([a])	8	11.5	.2	.2
1	23	31	4	4.5	.2	.2
0.5	8	10	3	3.5	.2	.2
0.2	6	7	3	3.5	.2	.2
0.1	6	7	3	3.5	.2	.2
0.05	5	5	2	2	.2	.2
Water, egg 1:1	3	3	2	2	.2	.2
Undiluted egg	4	4	2	2	.2	.2

[a] Not coagulated after 24 hours.

The data in table 9 show the time required for coagulation of egg albumin at 60°, 65°, and 100° C. when mixed with equal parts of various equimolal concentrations of dextrose and of sucrose. At 60° a material increase in the time required for coagulation was observed when the sugars were present in 1-molal or greater concentrations. When 3-molal and 4-molal solutions of either sugar were employed coagulation was prevented for at least 24 hours. It is especially significant that sucrose afforded more protection than dextrose. The albumin coagulated in 73 minutes at 60° in the presence of 2-molal dextrose, whereas an equimolal solution of sucrose prevented coagulation for 24 hours.

When the experiment was repeated at 65° and 100° C. much more rapid rates of coagulation were observed. For example, with the 3-molal solutions the albumin failed to coagulate in 24 hours at 60°, but when exposed to 65° coagulation occurred in 34 minutes in the dextrose and 52 minutes in the sucrose solutions. At 100° coagulation in all concentrations employed occurred in relatively few seconds.

Effect of Various Solutes on the Inactivation of Rennin by Heat

To a series of tubes each containing 2 cc of rennet extract was added an equal volume of one of the following substances: Conductivity water, 1-molal sodium chloride, 1-molal calcium chloride, 4-molal sucrose, and glycerol (specific gravity 1.25). The tubes, together with a control tube containing undiluted rennet, were placed in a water bath at 70° C. At various time intervals one drop of the rennet mixture from each tube was removed and added to tubes containing 10 cc of fresh, raw milk. The tubes of milk were held at room temperature and observed for the time of coagulation. Progressive inactivation of the rennin increased the time required to coagulate milk.

Table 10.—*Effect of various substances on the inactivation of rennin by heat*

[Material added to rennet, equal volume]

Period rennet mixture was exposed to 70° C. (minutes)	Coagulation time of 10 cc of milk by 1 drop of rennet mixture					
	Nothing added	Conductivity water	1-molal sodium chloride	1-molal calcium chloride	4-molal sucrose	Glycerol
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
0	34	30	29	20	33	20
2.5	40	72	41	22	32	24
5.0	109	(a)	319	22	31	31
7.5	162		(a)	36	27	36
10.0	314			39	25	100
12.5	550			101	61	106
15.0	(a)			109	70	135
20.0				230	65	260
30.0				594	55	(a)
45.0				(a)	40	
60.0					35	
100.0					50	
120.0					50	
150.0					44	
180.0					70	
240.0					55	
300.0					60	

[a] Not coagulated after 24 hours.

It will be observed from the data in table 10 that 1 drop of the undiluted rennet before heating induced coagulation of 10 cc of milk in 34 minutes. After the milk had been heated for 2.5, 5.0, 7.5, 10.0, and 12.5 minutes, the coagulation times increased to 40, 109, 162, 314, and 550 minutes, respectively. After exposure to 70° C. for 15 minutes the rennin was so completely inactivated that it could not induce coagulation in 24 hours. All the milk not coagulated after 24 hours was still sweet to the taste.

The rennet extract which was diluted with water was completely inactivated after 5 minutes' exposure. This agrees with the decreased thermal resistance observed with certain bacterial cells in water suspension. Likewise, it will be observed in table 10 that 4-molal sucrose very effectively protected rennin against destruction by heat. Even after 5 hours' exposure at 70° C. the rennin was sufficiently active to coagulate milk in 60 minutes. Glycerol and 1-molal calcium chloride also afforded definite protective action, although not to the same degree as sucrose. Sodium chloride apparently hastened the inactivation of the rennin. The data for suitable control tubes were not incorporated in the table as the coagulation times were identical with those observed for the undiluted rennet, except in the case of the milk to which calcium chloride was added. As might be expected, the addition of calcium chloride to the milk reduced the time of coagulation a few minutes in each case. The observations are so completely in accord with those made with cell suspensions that the possibility of the operation of the same set of factors is forcibly impressed.

DISCUSSION

The data in this paper lend support to the concept that the individual cell does not die suddenly after a given exposure to heat. Unfavorable environmental factors induce changes in the cells, which behave in accordance with well-established principles of colloidal chemistry. It is believed that all the salient features of the data presented can be explained on the basis of the theory of cell destruction outlined by Bancroft and Richter (*2*). This concept permits of a logical basis for elucidating protective action, increased growth in carbohydrate media, sensitivity of cells to water, and other observations made in connection with this study.

The degree of dispersion of the colloids of the normal cell presumably is at least nearly optimum for maximum stability. As the agglomeration of the colloids becomes progressively more pronounced, the cell becomes more sluggish and eventually dormant. Such a cell is narcotized and may be revived if placed under conditions conducive to peptization of the colloids to their normal degree of dispersion.

It is apparent that the more advanced the degree of coagulation, the greater will be the difficulty encountered in peptization. Similarly, one should expect decided differences in the peptizing qualities of various media. The coagulated colloids of an injured cell may be peptized by one medium and not by another. Obviously, reversibility of coagulation is a relative matter and depends upon a complementary relationship with the peptizing qualities of the medium employed.

A summary view of the data presented in this paper suggests that certain sugars retard the agglomerating action of heat on the protoplasmic colloids. As a result, cells subjected to a given heat treatment in concentrated sugar solutions are only reversibly coagulated, whereas in aqueous suspensions the protoplasm more closely approaches the irreversible stage of coagulation. In these experiments thermal exposures have been employed which were barely adequate to kill the cells in aqueous suspensions, thereby favoring a demonstration of the maximum protective action in the sugar suspensions. Experiments are now in progress with cultures whose heat resistance is just at the threshold of the thermal exposure of pasteurization. If some of the organisms in ice-cream mix are sufficiently protected by the presence of sugar to enable them to survive, the need for a readjustment of pasteurization requirements for this product would be suggested. The practical aspect of the protective action of sugar is also manifested in the production of sweetened condensed milk, and the preservation of foods containing high percentages of sugar such as fruits canned in sirup, honey, molasses, etc.

The protective action of hypertonic sugar solutions against the action of heat on egg albumin and rennin further suggests that the protection is afforded by retarding the rapid coagulation of the colloidal complex. The addition of water hastened the time of coagulation or destruction of the enzyme, and the addition of hypertonic sugar solutions retarded the same process. The parallelism between the observation with nonliving proteins and those made with living cells is quite apparent. The precise mechanism by which sugar solutions retard the coagulation of nonliving proteins is one which still challenges the attention of physical chemists. The data presented in this paper suggest that the same fundamental principles involved may apply to the destruction of bacteria.

When the cell colloids have agglomerated to the extent that all efforts fail to peptize them, the cell may be regarded, with reservations, as irreversibly coagulated or dead. The impracticability of proving such a point beyond all peradventure of doubt is illustrated by the many reports in the literature of delayed germination of heated cells. Although it is quite proper to conclude that the colloids of a cell are irreversibly coagulated with respect to a given medium, obviously the conclusion should be confined to the observation, especially if minimal exposures have been employed. Any determination of minimum lethal exposure is subject to question on the basis of the uncertainty of the death of the organism.

The importance of permeability in protective action is suggested by the results of several experiments. Prolonged exposure of certain cells to hypertonic solutions results in an actual increase in the number of cells capable of withstanding a given heat treatment, although the total number of cells in the unheated samples may show a steady decline. It is believed that this is explicable on a basis of different degrees and rates of permeability of the cells in the population. The variations observed in the protective action of various sugars for different organisms also suggest that penetration of the cell wall must play an important role in the regulation of protective action.

When certain cells are heated in a series of solutions of dextrose with increasing osmotic pressures, there is an unmistakable parallel increase in the protective action. A similar series of equimolal concentrations of sucrose will show considerably greater protective action than the dextrose solutions. This suggests that although osmotic pressure is an important factor in the protective action afforded by sugars it is not the only agency involved. If the protective action is to be explained on a basis of the transfer of water, obviously osmotic pressure would play an important part. On the other hand, the effective osmotic pressure at the cell surface is regulated not only by the molecules in solution but by the relative permeability at each individual cell surface.

SUMMARY AND CONCLUSIONS

This work involves a study of the increased resistance manifested by certain micro-organisms when heated in the presence of high concentrations of sugars. The significance of this phenomenon

in the heating of ice-cream mix is demonstrated and the probable relation to the preservation of condensed milk, canned fruits, honey, molasses, etc., is suggested.

Within the limitations imposed by the experimental procedure it has been possible to demonstrate some of the fundamental points bearing on the mechanism of this protective action. The permeability of the individual cell apparently plays an important part in regulating the rate of death in water and in sugar suspensions.

The protective action increased with increased osmotic pressure in a series of concentrations of a given sugar, but equimolal solutions of different sugars did not show the same protective action. Maltose and lactose gave little or no protection to the cells studied.

Not all cells exhibit the phenomenon of increased thermal resistacne in hypertonic solutions. Such resistance is believed to be limited to those cells which are highly sensitive to water at slightly increased temperatures. Washing of cells after exposure to hypertonic solutions removes any protective action which the sugar solution affords the cells in the unwashed portion.

Parallel with the protective action for cells, hypertonic sugar solutions have been shown to delay or even prevent the coagulation of egg albumin and to retard the inactivation of rennin by heat.

The general conclusions from these data may be briefly stated as follows: The addition of sugar to ice-cream mix may increase the thermal resistance of the microflora. Hypertonic solutions of dextrose and sucrose made up in broth, milk, water, or ice-cream mix afforded certain cells definitely greater protection against heat than when heated in water suspensions. The addition of sugar after heating did not induce an increased survival of the cells employed in this study. The variation in the peptizing qualities of different media for the cells which have been only reversibly coagulated by minimal exposures suggests caution in all studies based upon the death of the cell as measured by cultural methods.

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